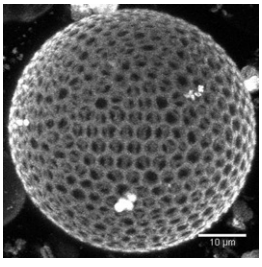
Membrane Physical Chemistry II

2731-Pos Board B717
Thermal Stress of Supported Lipid Bilayer Induces Formation and Collapse of Uniform Radius Tubules
Kimberly L. Weirich, D. Kuchnir Fygenson.
Lipid bilayer morphologies and the transitions between them are important to many cellular processes. Supported lipid bilayer (SLB) provides a model system in which to quantitatively investigate transitions from planar to tubular and tubular to spherical morphologies. Following a small increase in temperature (~5-10°C) flexible filaments extrude from a fluid SLB. Individual filaments can reach hundreds of microns in length before spontaneously collapsing into discs. We demonstrate that the filaments are tubular by decreasing the external buffer concentration, which causes them to swell, first into resolvable tubules with capped ends and then into giant vesicles. At high ionic strength, the sub-resolution tubules are adsorbed to the SLB, enabling the measurement of their radius to within ± 5 nm using conventional fluorescence microscopy. The radius depends on the lipid tail composition and varies $<10\%$ along the tubule length. Under tension, tubules are even more uniform, having no measurable variation in radius.

2732-Pos Board B718
Lipase Action on Self-Assembled Lipid Liquid Crystalline Nanoparticles
Justas Barauskas, Daniel Anderberg, Allan Svendsen, **Tommy Nylander**.
Natural lipase substrates are supramolecular assemblies, either emulsion droplets or liquid crystalline, aggregates. Most of the lipolysis takes place at interfaces and is dependent on the structure and organization of the lipid/water boundary as well as internal structure of the aggregate. The internal structure control both the access of the susbstrate as well as the ability to take care of the product. During the course of the reaction, hydrolysis products interact with lipid substrate, continuously change the interface and aggregate characteristics. Most of the studies so far have been focused on the changes of that take place at the lipid aqueous interface. We have used non-lamellar self-assembled lipid nanoparticles as well defined and biomimicing substrate in order to gain fundamental knowledge on lipase (Thermomyces lanuginosus lipase) catalyzed processes in terms of the changes in morphology and internal structure of the lipid aggregate (substrate). The results from two types of substrate structures will be presented, namely glycerol monooleate -based cubic and reversed hexagonal liquid crystalline nano-particles. These types nano-particles are likely to occur during the lipolysis process and also have potential use as drug delivery vehicles. The changes in lipid aggregate morphology and internal structure as a function of a lipase action have been investigated by means pH-stat titration, cryogenic transmission electron microscopy (cryo-TEM) and synchrotron X-ray diffraction techniques. In the cryo-TEM study we also used lipase conjugated to gold nanoparticles, enzymatically active hybrid nanoparticles, to simultaneously visualize the location of the enzyme and the effects of enzymatic digestion of lipid aggregates. Our results clearly show that the enzyme action is strongly influenced by the self-assembled structure and the lipid composition providing the possibility to control lipase activity.

2733-Pos Board B719
Vesicle and Lipid Bilayer Dynamics: Cross-Linking Effects and FRAP Analysis
Michael Kessler, Robin Samuel, Katerine Baldwin, Rahul Gupta, Arthur Lee, Susan D. Gillmor.
We investigate the effects of perturbations on lipid phase dynamics. Our primary tools are confocal microscopy and differential scanning calorimetry (DSC). In coexisting fluid two-phase vesicles we have characterized cross-linking in the fluid disordered phase. Instead of reaching thermodynamic equilibrium, we have documented an increase in meta-stable configurations. Using fluorescence recovery after photobleaching (FRAP), we investigate how cross-linking affects diffusion in lipid bilayer. The diffusion perturbations reveal that cross-linking and non-specific binding slows lateral mobility, which alters lipid dynamics. Since cell membranes are not at thermodynamic equilibrium, our investigations into the dynamics behavior are pertinent to understanding membrane response to common events, such as receptor-ligand complexing, glycosylation, and receptor platform formation.



2734-Pos Board B720
Modulated Phases in 4-Component Lipid Mixtures
Jonathan J. Amazon, Jing Wu, Frederick A. Heberle, Gerald W. Feigenson.
The 3-component lipid mixture DSPC/POPC/CHOL exhibits a rich equilibrium compositional phase diagram. When a fourth lipid component, DOPC, is titrated in to replace POPC, unusual phase separation emerges in a portion of the composition space. Elongated stripe and honey comb like structures of coexisting liquid ordered and liquid disordered phases are observed in giant unilamellar vesicles. GUV images were examined by fluorescence wide field, 2-photon, and confocal microscopies. The specific mechanisms by which this phase modulation is driven are not yet well understood. The work of Seul and Andelman suggests that the formation of these structures may be driven by multiple order parameters with competing interactions. We analyzed many different order parameters and interactions using the formalism of field theory, diffusion kinetics, and computational modeling to better characterize the microscopic details of this novel phase behavior.

2735-Pos Board B721
Dynamic Critical Exponent for Concentration Fluctuations in a Lipid Bilayer
Aurelia R. Honerkamp-Smith, Benjamin B. Machta, **Sarah L. Keller**.
We present the first systematic measurement of the effective dynamic critical exponent z(eff) in a 2-dimensional system with conserved order parameter surrounded by a bulk 3-dimensional fluid, here a lipid membrane in water. We measure the dynamic structure function for concentration fluctuations in the membrane. We use dynamic scaling to collapse structure functions at different wavenumbers, thereby obtaining the effective dynamic exponent z(eff). We find that as the membrane approaches the critical temperature, z(eff) approaches 3, consistent with theoretical prediction [1]. Our result is fundamental to both biology and physics since membranes isolated from cells are poised near miscibility critical points [2].
References
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2736-Pos Board B722
Miscibility of Ternary Membranes Containing Charged Lipids and Confirmation of Membrane Composition by Mass Spectrometry
Matthew C. Blosser, Cameron W. Turtle, Jordan B. Starr, Sarah L. Keller.
Here I present phase diagrams of vesicles containing phosphatidylcholine (PC), an uncharged lipid; phosphatidylglycerol (PG), a charged lipid; and cholesterol. I have found that this mixture exhibits coexisting liquid phases over a wide range of temperatures and compositions. I have found that miscibility in membranes containing charged lipids occurs over similar ranges of temperatures and lipid compositions as in membranes containing only uncharged lipids. Techniques for creating vesicle membranes containing charged lipids are significantly more challenging and less well characterized than for membranes containing neutral lipids. Here I use mass spectrometry to determine that the final membrane lipid composition is close to that of the initial stock solution. Specifically, I use a quadrupole spectrometer with an electrospray source using multiple reaction monitoring (MRM) in both positive and negative mode. Since the same instrument is used to quantify both the ratio of PC to PG and PC to cholesterol, the entire composition can be determined from one sample. The sensitivity of the technique is higher than phosphorus assays, and the MRM makes it extremely unlikely that signal is due to molecules other than the lipids of interest. This result confirms that vesicles made by gentle hydration have a predictable composition at both high and low fractions of charged lipids.

2737-Pos Board B723
Measurement of Late Stage Coarsening on Lipid Membranes
Cynthia A. Stanich, Aurelia R. Honerkamp-Smith, Gregory Garbès Putzel, Thien-An D. Hua, Andrea K. Lamprecht, Christopher S. Warth, Sarah L. Keller.
We investigate the diffusion and growth of liquid domains in the membrane of giant unilamellar vesicles (GUVs) composed of a ternary mixture of saturated phospholipids, unsaturated phospholipids, and cholesterol when the temperature is quenched below the miscibility transition temperature. After a period of nucleation (Lifshitz, et al., 2002), domains can grow by two mechanisms in the late stages of coarsening. These mechanisms are collision and coalescence of liquid domains and Ostwald ripening. Both contribute to the measured growth exponent, z, where domain radius, $R \propto t^z$. If the area fraction of one of the phases is small, the later stage of domain growth has been predicted by

simulation and theory to be z = 1/3 (Laradji & Sunil Kumar, 2004). When the area fraction is increased to 50% of the surface of the membrane, simulation predicts $R \propto t^{1/2}$ (Fan et al., 2010; Laradji & Sunil Kumar, 2005). Here we compare physical measurement of the growth exponent to theory in both cases. We also show preliminary evidence of Ostwald ripening on GUVs.

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2738-Pos Board B724

Endophilin N-Bar Domain is Sorted by Membrane Curvature in a Solution Concentration-Dependent Manner

Chen Zhu, Tobias Baumgart.

Membrane curvature provides an active means to control spatial organization and activity of cells and is regulated and explored by a plethora of peripheral membrane proteins, including dynamin, as well as proteins containing BAR domains and amphiphatic membrane-binding helices. The protein endophilin has been shown to be involved in curvature-sorting phenomena during clathrin-mediated endocytosis in neuronal synapses. The mechanisms underlying its curvature-sorting are currently unclear. In addition to scaffolding effects contributed by its BAR domain and N-terminal hydrophobic helix membrane insertion, oligomerization likely contributes to membrane curvature sensing and generation.

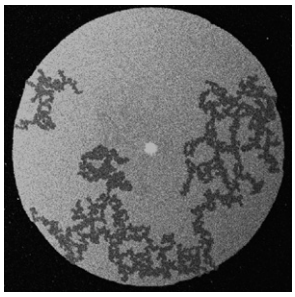
In order to enhance the biophysical understanding of membrane curvature sorting, we are using the following approach. Highly bent cylindrical membrane tethers are pulled from pipette-aspirated giant vesicles. Fluorescence images revealed that rat A1 endophilin N-BAR domains (E-N-BAR) preferentially partitioned onto the tethers rather than the low curvature vesicles. We quantified the sorting of E-N-BAR via image analysis of confocal microscopy tether cross section images. We found that curvature-sorting of E-N-BAR characteristically depends on aqueous solution protein concentrations. Ratiometric assessments of curvature-partitioning at two different solution concentrations furthermore revealed nonlinear trends in curvature composition coupling. We also developed a new thermodynamic curvature/composition coupling model to analytically interpret our measurements. In addition, our findings from fluorescence photobleaching recovery (FPR) measurements showed that diffusion coefficients of E-N-BAR on tethers decrease with increasing membrane curvature, revealing curvature-dependent molecular crowding consistent with our curvature sorting theory.

2739-Pos Board B725

Fractal Avalanche Ruptures in Biological Membranes

Irep Gözen, Paul Dommersnes, Ilja Czolkos, Aldo Jesorka,
Tatsiana Lobovkina, Owe Orwar.

Bilayer membranes envelope cells as well as organelles; constitute the most ubiquitous biological material found in all branches of the phylogenetic tree. Cell membrane rupture is an important biological process and substantial rupture rates are found in skeletal and cardiac muscle cells under mechanical load. Rupture can also be induced by processes such as cell death, and active cell membrane repair mechanisms are essential to preserve cell integrity. Pore-formation in cell membranes is also at the heart of many biomedical applications such as in drug, gene, and siRNA delivery. Membrane rupture dynamics has been studied in bilayer vesicles under tensile stress, which consistently produce circular pores. We observed very different rupture mechanics in bilayer membranes spreading on solid supports: in one instance fingering instabilities were seen resulting in floral-like pores; in another, the rupture proceeded in a series of rapid avalanches causing fractal membrane fragmentation. The intermittent character of rupture evolution and the broad distribution in avalanche sizes is consistent with crackling-noise dynamics. Such noisy dynamics appear in fracture of solid disordered materials, in dislocation avalanches in plastic deformations, domain wall magnetization avalanches. We also observed similar fractal rupture mechanics in spreading cell membranes.



2740-Pos Board B726

Giant Unilamellar Vesicles with Internalized Poly-(n-Isopropylacrylamide)-Vinyl Ferrocene Copolymer (PNIPAAm-VFC), a New Membrane-Interactive Thermoresponsive Material

Ilona Wegrzyn, Birgit Nagel, Martin Katterle, Owe Orwar, Aldo Jesorka.

It is very challenging to develop artificial systems mimicking components of the biological cell, but offers rewards with respect to a better understanding of cell processes, function and complexity. Giant unilamellar vesicles are micro-sized biomimetic compartments. They possess a simple, very important structural feature of natural cells, the double layer membrane, but are otherwise limited in internal functionality. There are a few existing methods of introducing more complex internal structure into liposomes. Internalizing water soluble polymers like poly-*N*-isopropylacrylamide (PNIPAAm) or poly-ethylene glycol (PEG) is a suitable method to increase concentration, viscosity, and to achieve compartmentalization, thus approaching more complex artificial cell architectures [1-3]. This time we want to present a new type of thermoresponsive polymer, PNIPAAm with co-polymerized vinyl ferrocene (VFc)[4]. The increased hydrophobicity achieved by 3% (mass) ferrocene, promised better dynamic properties, reduced equilibrium compartment size and more homogenous hydrogels. During our investigation, an exceptionally strong interaction between PNIPAam-VFc and the vesicle boundary was observed. Polymer chains are anchored in the bilayer membrane by means of the lipophilic metallocene groups, creating unexpectedly strong attachment points. After increasing the temperature above the lower critical solution temperature where gel formation sets in, numerous lipid nanotubes are pulled from the vesicle, connecting the vesicular membrane with the internal hydrogel compartment surface. This leads to spontaneous shape changes of the vesicle, associated with multiple protrusion formation, followed by rapid coarsening to a single liposome.

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2741-Pos Board B727

Probing Membrane-Surface Interactions via Brownian Motion of Micro-Sized Beads

Xiaojian Chen, Dong Gui, Nancy Bernal, Eugina Olivas, Hector Garcia,
Shane Nystrom, Leonid Pryadko, Roya Zandi, Umar Mohideen.

Interactions between a free standing bilayer lipid membrane and various surfaces have been investigated by monitoring the Brownian motion of micron-sized silica beads on a GMO membrane. This membrane was suspended across a 200 μm Teflon aperture in either water or buffer solution using the painting method. Silica beads with diameters ranging from 0.7 μm - 5 μm were spread on the membrane and their motions were recorded at a rate of 10 frames/sec. The beads were either left unfunctionalized after cleaning or further functionalized with various chemical groups, including amine, PEG, methyl, octadecyl, avidin as well as the membrane itself. Since the interface interactions between the membrane and the beads leads to increased friction, it would be detected if the bead slowed in comparison with free Brownian motion. Experiments showed that the membrane functions as a near-perfect surface, lacking non-specific interactions. In all cases, only the avidin-biotin and membrane-membrane interactions lead to retarded motion and only in a buffered solution. This study on the Brownian motion of micron-sized beads on a membrane provides a convenient novel probe to detect interface interactions. As the beads used in these experiments have similar sizes to most bacteria and cells, they can be treated as bacteria/cell analogues and used to simulate the adsorption of bacteria /cells on another membraned bioorganism.

2742-Pos Board B728

Phospholipid Bilayers are Viscoelastic

Christopher W. Harland, Tristan T. Hormel, Miranda J. Bradley,
Raghuveer Parthasarathy.

The two-dimensional fluidity of lipid bilayers is crucial to biological function, as it enables the mobility of membrane macromolecules. Though the existence of membrane fluidity is well established, its fundamental nature remains poorly characterized, and many models of membrane dynamics implicitly or explicitly assume that the lipid bilayer is a simple Newtonian liquid. Three-dimensional fluids as diverse as chocolates and cytoskeletal networks show a rich variety of Newtonian and non-Newtonian dynamics that have been illuminated by recently developed rheological techniques. Applying particle tracking microrheology to freestanding phospholipid bilayers, we find that the membranes are not simply viscous liquids but rather exhibit viscoelasticity, with an elastic modulus that dominates the response above a characteristic frequency that diverges at the fluid-gel (La-Lb) phase transition temperature. These findings fundamentally alter our picture of the nature of lipid bilayers and the mechanics of membrane environments.